

Functional evaluation for effective compositions in seed oil of Korean pine

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Abstract: The effect of seed oil of Korean pine (*Pinus koraiensis*) on the rats' blood-fat and its anti-ageing function was studied for appraising the efficacy of the seed oil of Korean pine. Sixty experimental rats were randomly divided into 5 groups (half males and half females in each group) as normal control group, high fat diet control group, and three groups (Group 1 Group 2, Group 3) that were fed with feedstuff with the contents of the seed oil of 2.0g/(kg·d⁻¹), 4.0g/(kg·d⁻¹) and 8.0g/(kg·d⁻¹), respectively. The indexes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and anti-oxidation capacity (AOC) were measured by Reagent Kit method. It was found that the seed oil of the Korean pine could reduce the content of triglyceride and improve SOD as well as GSH-PX activity in serum. These indexes of the rats in Group 2 fed with Korean pine seed oil of 4.0 g/(kg·d⁻¹) reached the significant level and those of rats in Group 3 fed with the seed oil of 8.0 g/(kg·d⁻¹) reached the extremely significant level. The results indicated the seed oil of Korean pine had function of regulating the level of blood-fat and anti-ageing.

Keywords: Korean pine seed oil; Effective compositions; Functional evaluation; Blood-fat; Anti-ageing

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Introduction

The Korean pine (*Pinus koraiensis*) distributed in the northeastern China is one of precious tree species. Its seed was regarded as not only medicine but also food, and it was favored extremely by doctor and nutritionist in China (Yu *et al.* 2001). As a functional product, it has an extensive application prospect in the field of food industry (Yue *et al.* 1999; Rao *et al.* 2002). Korean pine seed oil contains abundant biological and active materials that have important functions of regulating human physiological activity. In these years, there were few studies on the functions of seed oil of Korean pine (Guo *et al.* 1996; Guo *et al.* 2001). This study evaluated the functions of the seed oil of Korean pine on the rats' blood-fat and its anti-ageing function, aimed at providing scientific basis for utilization of seed oil of Korean pine.

Materials and methods

Materials

The seed oil of Korean pine was obtained by supercritical fluid extraction. Wistar rats that were all 22-month old (30 males, 30 females) were provided by Harbin Animal Science and Veterinary Medicine Institute, Chinese Academy of Agricultural Sciences. Reagent kits of BG (blood glucose), HDL-C (high-density lipoprotein-cholesterol), LDL-C (low-density lipoprotein-cholesterol), TC (cholesterol) and TG

(triglyceride) were provided by Zhong-sheng Bei-kong Biotechnology and Science Inc. Reagent kits of MDA (malonic aldehyde), SOD (superoxide dismutase), GSH-PX (glutathione peroxidase) and AOC (anti-oxidation capacity) were provided by Nanjing Ji-ancheng Bioengineering Institute.

Methods

Sixty experimental rats were randomly divided into 5 groups (half males and half females in each group) as normal control group, high fat diet control group and three groups fed by Korean pine seed oil. The rats in normal control group were fed with common feedstuff every day. The rats in high fat diet control group were fed with high fat diet feedstuff (90% common feedstuff, 1% TC, 8.8% lard, 0.2% bile salt). The rats in group 1, group 2, and group 3 were fed with feedstuff containing Korean pine seed oil of 2.0 g/(kg·d⁻¹), 4.0 g/(kg·d⁻¹) and 8.0 g/(kg·d⁻¹), respectively during the experiment. The level of BG, HDL-C, LDL-C, TC, TG in serum after 28-day treatment, the activity of SOD, GSH—PX, AOC and the content of MDA in serum after 56-day treatment were determined by Hitachi 7020 Automatic Chemistry Analyzer (Japan) and 752 Spectrophotometer (Shanghai China). The data were expressed as Mean±Std by Deviation Statistical Analysis (SPSS 11.0).

Results

Effect of Korean pine seed oil on blood glucose and lipid

The lipid metabolism of rats in high fat diet control

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group presented disorder and the level of serum TC increased remarkably ($P<0.05$), compared with those in the normal control group (Table 1). The level of serum TC increased and the level of serum TG decreased, especially in group fed by the Korean pine seeds oil for 28 days. The dif-

ference of the level of BG, HDL-C was not remarkable in different groups ($P>0.05$), but LDL-C in group 3 fed by the Korean pine seed oil of $8.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$ was significantly increased compared to the high fat diet group (Table 1).

Table 1. Effect of seed oil of Korean pine on BG and level of blood lipid of rats

Group	BG	TC	TG	HDL-C	LDL-C
Normal control group	4.32 ± 1.33	1.73 ± 0.18	0.81 ± 0.23	0.86 ± 0.05	0.40 ± 0.10
HFD control group	5.21 ± 1.24	2.54 ± 0.70^a	0.91 ± 0.44	0.76 ± 0.17	0.52 ± 0.15
Group 1	4.73 ± 1.50	2.73 ± 0.59^{aa}	0.66 ± 0.10	0.77 ± 0.18	0.48 ± 0.17
Group 2	4.23 ± 0.64	2.52 ± 0.48^a	0.62 ± 0.07^b	0.75 ± 0.20	0.68 ± 0.29^a
Group 3	5.89 ± 1.33	2.78 ± 0.39^{aa}	0.74 ± 0.17	0.67 ± 0.23	$0.78 \pm 0.13^{aa/b}$

Notes: Group 1 is for rats fed with feedstuff containing Korean pine seed oil of $2.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$; Group 2 is for the rats fed with feedstuff containing Korean pine seed oil of $4.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$; Group 3 is for rats fed with feedstuff containing Korean pine seed oil of $8.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$. HFD control group means high fat diet control group. Compared with normal control group, "a" means the remarkable difference ($P<0.05$), and "aa" means the extremely difference ($P<0.01$). Compared with HFD control group, "b" means the remarkable difference ($P<0.05$). The rats in this experiment are all male.

Effect of seed oil of Korean pine on male rats' weight

The increased weight of the rats in Group 2 and Group 3 was much less than that of the rats in HFD group, and the difference was remarkable ($P<0.01$) (Table 2, Fig. 1 and Fig. 2). But the group1 fed by Korean pine seed oil of $2.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$ increase significantly on weight ($P>0.05$),.

The increase weight of HFD control group was much more than that of normal control group. The increase weight of the Group 1 was as much as that of HFD control group. But the increase weights of Group 2 and Group 3 were even much lower than that of normal control group ($P>0.05$).

Table 2. Effect of Korean pine seed oil on male and female rats' weight

Group	Male			Female		
	Average weight/g		Increase weight/g	Average weight/g		Increase weight/g
	Before treatment	After treatment		Before treatment	After treatment	
Normal control	526.67	546.00	19.33	270.00	291.67	21.67
HFD control	495.00	540.00	45.00	296.67	330.00	33.33
Group 1	505.00	546.67	41.67	295.00	324.00	29.00
Group 2	511.67	521.67	10.00^{aa}	296.67	312.00	15.33^{aa}
Group 3	508.33	523.33	15.00^{aa}	298.33	306.00	7.67^{aa}

Notes: "aa" means the extremely difference ($P<0.01$). The meaning of group 1, 2 and 3 is as same as Table 1.

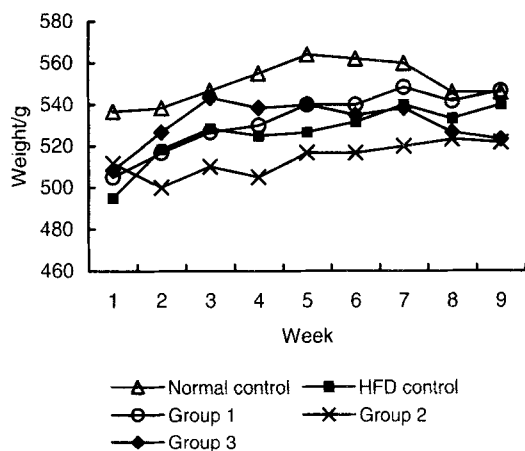


Fig. 1 Effect of Korean pine seed oil on increase weight of male rats

Note: Group 1, Group 2 and Group 3 were fed with Korean pine seed oil of $2.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$; $4.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$; $8.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$ respectively.

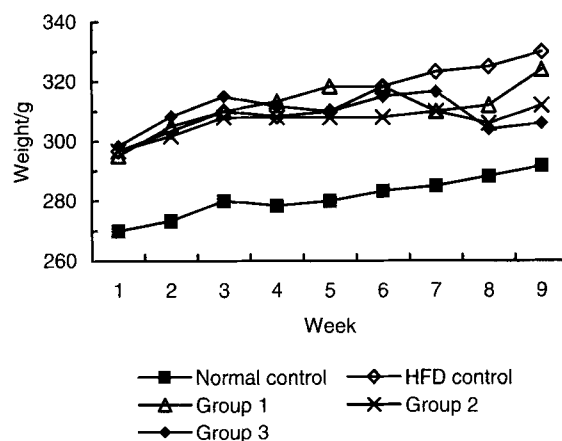


Fig. 2 Effect of Korean seed oil on increase weight of female rats

Group 1, Group 2 and Group 3 were fed with Korean pine seed oil of $2.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$; $4.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$; $8.0 \text{ g}/(\text{kg} \cdot \text{d}^{-1})$ respectively.

From Fig. 1, the weight of male rats in the normal control group increased to the top at 5th week, and then decreased slowly. For HFD control group, Group 1 and Group 2 the weight of male rats increased slowly all the time. In the Group 3, male rat weight reached the top on 3rd week, and then decreased slowly.

Effect of seed oil of Korean pine on female rats' weight

From Table 2 and Fig. 2, we can see that the female rats in HFD group had the most significant increase in weight. The increase weights of Group 2 and Group 3 were lower than

that of HFD group. And the increase weight of female rats was less than that of male rats (Table 2).

Effect of seed oil of Korean pine on serum MDA, SOD, GSH-PX and AOC

SOD, GSH-PX activity and AOC of rats in Group 2 and Group 3 were significantly increased ($P<0.05$ or $P<0.01$), compared with that of HFD control group, while the serum MDA content was significantly decreased by the oil treatment for 8 weeks ($P<0.05$ or $P<0.01$).

Table 3. Effect of seed oil of Korean pine on serum MDA, SOD, GSH-PX and AOC

Group	MDA /nmol · mL ⁻¹	SOD /U · mL ⁻¹	GSH-PX /U · mL ⁻¹	AOC /U · mL ⁻¹ (serum)
Normal control	9.37±0.71	128.46±11.11	208.72±28.35	14.04±1.14
HFD control	10.03±0.70	109.22±9.28	162.29±24.41	12.23±1.15
Group 1	9.45±1.52	110.32±13.11	173.73±14.21	13.13±1.31
Group 2	8.76±1.15 ^a	121.06±12.72 ^a	191.90±19.24 ^{aa}	14.82±1.41 ^{aa}
Group 3	7.96±1.34 ^{aa}	125.57±7.66 ^{aa}	202.01±13.75 ^{aa}	14.60±1.38 ^{aa}

Notes: Group 1 is for rats fed with feedstuff containing Korean pine seed oil of 2.0 g/(kg·d⁻¹); Group 2 is for the rats fed with feedstuff containing Korean pine seed oil of 4.0 g/(kg·d⁻¹); Group 3 is for rats fed with feedstuff containing Korean pine seed oil of 8.0 g/(kg·d⁻¹). HFD control group means high fat diet control group. "a" means the remarkable difference ($P<0.05$), and "aa" means the extremely difference ($P<0.01$), compared with HFD control group.

Discussion

It is reported that free radical is not only the pathological basis of many diseases or trauma, but also a main cause for accelerating organs ageing. The accumulation of the radicals is responsible for some damage of cellular constituents, such as the devitalization of membrane bound enzymes, DNA strand broken, peroxidation of biomembrane phospholipid with the resultant alteration by ion permeability and mitochondrial dysfunction. The over-production of free radicals attributed to the abnormal stimulation of lipid peroxidation (Zhao *et al.* 2000; Wang *et al.* 2002). Our study showed that the seed oil of Korean pine decreased the TG content significantly, but had no effect on TC, HDL-C. The reason in terms of free radicals still needs to be further studied.

It is well known that MDA is the production of lipid peroxidation. SOD and GSH-PX are the most important free radical scavenger. The results in this study showed that the seed oil of Korean pine could significantly decrease the level of serum lipid peroxidation, MDA content and improve the activities of SOD, GSH-PX and AOC. So, the seed oil of Korean pine has the function of regulating the level of serum lipid and anti-ageing in rats to some extent.

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